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DRUG DISCOVERY

Anti-inflammatory activity of the methanol leaf extract and fractions of *Lannea barteri* Oliv. Engl. (Anacardiaceae) in rats

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ABSTRACT

The *L. barteri* leaf extract and fractions were assessed for their anti-inflammatory potentials. The extract (200 and 400 mg/kg) and fractions (400 mg/kg)were employed in egg albumin-induced edema while 3% Tween 80 and piroxicam (50 mg/kg) served as negative and positive controls respectively. In xylene induced ear edema, 100 mg/ear of the extract, fractions and piroxicam was used while 3% Tween 80 served as the negative control. In cotton pellet-induced granuloma, 200 and 400 mg/kg extract and fractions, diclofenac 4 mg/kg and 3% Tween 80 were used. The HFLB caused the highest (40%) edema reduction at 3rd hour while

200 mg/kg ME caused the highest (28%) reduction at the 5th hour egg albumin-induced edema. There were significant ($p<0.05$) reduction of ear edema induced by xylene across the various groups compared to control. The HFLB caused the highest significant ($p<0.01$) percentage reduction (42.59%) of the granuloma tissue formed when compared to control. This study established that the methanol leaf extract and fractions of *L. barteri* reduce inflammation validating the folkloric use of *L. barteri* for the treatment inflammation and pain.

Keywords: Anti-inflammatory activity, Egg albumin, Granuloma, *Lannea barteri,* Piroxicam

1. INTRODUCTION

Inflammation and pains are usually symptoms implicated in many various clinical presentations irrespective of the causative factors (Dhara et al., 2000). The reaction to these phenomena is characteristic responses by all living tissues. It is complex and is brought about by various mediators such as histamine, prostaglandins E2 and I2 that are synthesized as a result of the interaction of the noxious substances or micro-organisms with the tissue (Barnes and Karin, 1998; Ganesh et al., 2008). Release of cascade of inflammatory mediators makes inflammatory reaction to involve both vascular and cellular components (Luster, 1998; Akuodor et al., 2015). Anti13 Many current anti-inflammatory drugs target these mediators at differentlevels, yet they lack specificity and their untoward effectsrestrict their long-term use (Dhikav et al., 2002). Hence, there is a constant demand for better therapeutic alternatives.

Lannea barteri is often harvested from the wild for local use. A red-brown dye from this plant is traditionally used in dyeing in Africa while other parts are used as food, medicines and wood work (Garba *et al.,* 2015). The plant is of ethnomedicinal relevance. The bark is stomachic. A decoction taken orally is used to treat gastric pains, diarrhoea, oedema, paralysis, epilepsy and madness (Kone et al., 2011). Combination of this plant with some spices (Yoruba, species) is useful as vermifuge. Externally, the bark is used to treat sores, ulcers, and leprosy (Jansen, 2005) and a root decoction cures hernia. The root, when ground, wrapped in the leaves of an unknown species can be used as a poultice on wounds. A leaf decoction is a remedy for haemorrhoids (Jansen, 2005). In northern Nigeria, the stem bark of *L. barteri* is used in the treatment of epilepsy, gastritis, childhood convulsions for the past decades among other uses (Garba et al*.,* 2015). The efficacy of this plant is generally well known among the traditional medicine practitioners. Scientific investigations on this plant have validated some of these claims such as antioxidant, acetylcholinesterase inhibitory effect, antibacterial (Kone et al., 2011), anticonvulsant (Garba et al., 2015) activities. However, there is still no documented research on its wound healing and anti-inflammatory potentials. This study therefore, evaluated the wound healing, anti-inflammatory and antimicrobial potentials of *L. barteri* leaf extract and fractions in rats.

2. MATERIALS AND METHODS

Equipment

Plethysmometer, sensitive weighing balance (Ohaus, Germany), surgical instrument, animal weighing balance (Griffin and George, London), bulk balance, animal cages, etc.

Chemicals, drugs and solvents

All the chemicals, reagents and solvents used were of analytical grade. They include methanol (Fluka, England), 3% Tween 80(R), piroxicam, diclofenac, xylene, chloramphenicol, ketamine, egg albumin, soft white paraffin wax and chloroform.

Animals

In-bred Swiss albino rats of either sex (170 - 220 g) were obtained from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The animals were kept in metal cages in the department's animal house facility and were freely maintained with food and water. The animals were handled according to the international guidelines on the use of laboratory animals.

Plant material

Fresh leaves of *Lannea barteri* were collected from the forest of Benue State, identified and authenticated by Mr Alfred Ozioko (a plant taxonomist) of International Centre for Ethnomedicine and Drug Development (Inter-CEDD), Nsukka, Nigeria. The specimen voucher with No 096 was deposited in the centre's herbarium.

Preparation, extraction and fractionation of plant material

The fresh leaves of *L. barteri* were air-dried, pulverized into coarse powder, weighed and stored in an air-tight container. The powder material (2000 g) was macerated with 10 litres of methanol for 72 h with intermittent agitation. After the extraction, the sample was filtered using Whatman no. 4 filter paper and the filtrate concentrated using rotary evaporator at a temperature of 50°C to obtain methanol leaf extract (MELB). The weight and the percentage yield of the dried extract were determined and the extract was stored at 4°C refrigerator for later use.

The MELB (100 g) was fractionated successively in a column chromatography using the solvents; n-hexane, ethyl acetate and methanol in the increasing order of polarity to obtain the n-hexane (HFLB), ethylacetate (EFLB) and methanol (MFLB) fractions. The fractions were evaporated to dryness using rotary evaporator at a temperature of 50°C and their percentage yields determined. They were stored separately in amber coloured bottles and preserved in the refrigerator.

Acute toxicity test (LD_{50})

The method described by Lorke (1983) was used to determine the acute intoxication and lethality of MELB in mice through the intraperitoneal cavity.

Phytochemical screening

The methods of Harborne (1973) and Trease and Evans (1989) were used to screen the extract and fractions for the presence of bioactive compounds.

Topical mouse ear edema induced by xylene

Using the methods of Tubaro et al. (1985) and Atta and Alkohafi (1998) with some modifications, the effects of extract and fractions on topical acute inflammation were evaluated. Swiss albino mice (19 – 35g) of both sexes were allotted into 6 groups of 6 animals per group. The treatment group were given methanol extract, ethylacetate fraction, n-hexane fraction, methanol fraction of (5 mg/ear) applied on the anterior surface of the right ear with 3% Tween 80 (0.05 ml/ear) and piroxicam (0.05 mg/ear) serving as negative and positive controls respectively. The anti-inflammatory activity was evaluated based on percentage edema reduction in the treated animals as against negative control animals using the relation:

$$\text{Percentage inhibition} = \left[1 - \left(\frac{R_{t-L_t}}{R_{c-L_c}}\right)\right] \times 100$$

Where R_t = mean weight of right ear plug of treated animals; L_t = mean weight of left ear plug of treated animals; R_c = mean weight of right earplug of control animals; L_c = mean weight of left earplug of control animals.

Egg albumin-induced rat paw edema

To assess the sub-acute systemic edema reducing effect of the extracts and fractions, the rat paw edema method (Winter et al., 1962) was used by measuring the acute inflammation in terms of change in volume of edema of the rat hind paw (Backhouse et al., 1996) induced by injection of egg albumin into the subplantar region of the rats' right hind paw. Inflammation was assessed as the variation between the zero-time volume of the treated paw (V_0) and the volume at the various times (V_t) after the injection of the phlogistic agent. Calculation of the percentage inhibition of edema (Ahmed., 1993; Perez, 1996) was done via the relation.

$$\text{Inhibition of edema (\%)} = \left[1 - \left(\frac{a-x}{b-y}\right)\right] \times 100$$

Where, a = mean paw edema volume of treated animals after egg albumin injection;
x = mean paw edema volume of treated animals before egg albumin injection
b = mean paw edema volume of control animals after egg albumin injection
y = mean paw edema volume of control animals before egg albumin injection

Cotton pellet induced granuloma in rats

To create the dead space wound, the method described by Rathi et al. (2004) was followed. Ten groups of rats with 6 animals per group received treatments orally as follows: groups A and B (200 mg/kg and 400 mg/kg of MELB in 3% Tween 80 respectively), Group C and D (200 mg/kg and 400 mg/kg of HFLB in 3% Tween 80 respectively), Groups E and F (200 mg/kg and 400 mg/kg of

EFLB in 3% Tween 80 respectively), Groups G and H (200 mg/kg and 400 mg/kg of MFLB in 3% Tween 80 respectively) and Groups I and J (diclofenac 4 mg/kg and 5 ml/kg of 3% Tween 80 respectively) for 9 days. Weight of granuloma in comparison to control group after drying to a constant weight at 60 ^{0}C was calculated thus:

Weight of granuloma = Dry weight of the granuloma containing cotton - weight of the cotton pellet

Statistical analysis

Data obtained were analyzed using Graph Pad Prism 5.03 and subjected to Dunnett's post hoc test. Results were expressed as mean ± standard error of mean and differences between means of treated and control groups were calculated at 95% confidence interval and accepted significant at $p < 0.05$.

3. RESULTS

Extraction

The percentage yields of extract and fractions were 164 g (8.2%) MELB, 14.62 g (14.62%) HFLB, 9.36 g (9.36% w/w) EFLB, 20.54 g (20.54%) MFLB.

Acute toxicity test

Oral administration of MELB, HFLB, EFLB and MFLB suspended in 3% Tween 80 in rats up dose of 5000 mg/kg caused no death or any observable sign of acute intoxication.

Phytochemical screening

Preliminary qualitative phytochemical analysis of the extract and fractions gave positive reactions for steroids, glycosides, saponins, flavonoids, alkaloids, reducing sugar, terpenoids, proteins, tannins, carbohydrates, resins and fat and oil (Table 1).

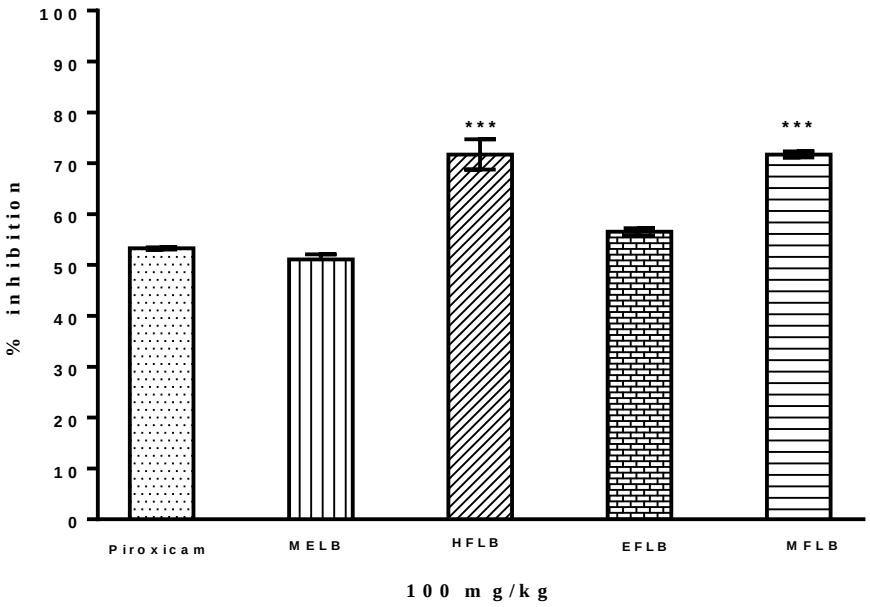
Table 1 Phytoconstituents of extract and fractions of *L. barteri*

Constituents	ME	HF	EF	MF
Alkaloids	++	-	-	+++
Carbohydrates	++	-	-	+++
Fats and oil	++	+	-	-
Flavonoids	+++	-	-	+++
Glycosides	+++	-	-	++
Proteins	+++	-	-	+
Reducing sugars	+	-	-	++
Resins	++	+	++	+
Saponins	+	-	-	++
Steroids	++	+	-	+++
Tannins	++	-	-	++
Terpenoids	+++	+	+++	-

Key: - = absent, + = present, ++ = moderately present, +++ = abundantly present, ME = methanol extract; HF = n-hexane fraction; EF = ethylacetate fraction; MF = methanol fraction.

Topical ear edema

The topical ear edema caused by xylene as shown in Fig 1 was inhibited by the extract and fractions. The HFLB caused the highest inhibition of the edema with a value of 78.26% followed by MFLB (71.74%), and EFLB (56.52%) showing that they had better activity than the positive control (piroxicam) with 53.26% and the MELB with 51.09% inhibition at the same concentration.



Values are mean ± SEM.; n = 5; *** Significant difference from control ($p < 0.001$) respectively; MELB = methanol extract; HFLB = n-hexane fraction; EFLB = ethylacetate fraction; MFLB = methanol fraction.

Figure 1 Effect of extract and fractions of *L. barteri* on topical ear edema

Egg albumin induced edema

Oral administration of extract caused a dose-dependent inhibition of egg albumin induced rat paw edema. In the first phase (3 h) of inflammation, HFLB caused highest inhibition (40%) of the edema formed followed by MELB (200 and 400 mg/kg) which caused 30% inhibition while in the second phase (5 h), MELB (200 mg/kg) caused highest inhibition (33%) followed by EFLB that caused 19.3% inhibition (Table 2).

Table 2 Effects of the extract and fractions of *L. barteri*on systemic rat paw edema

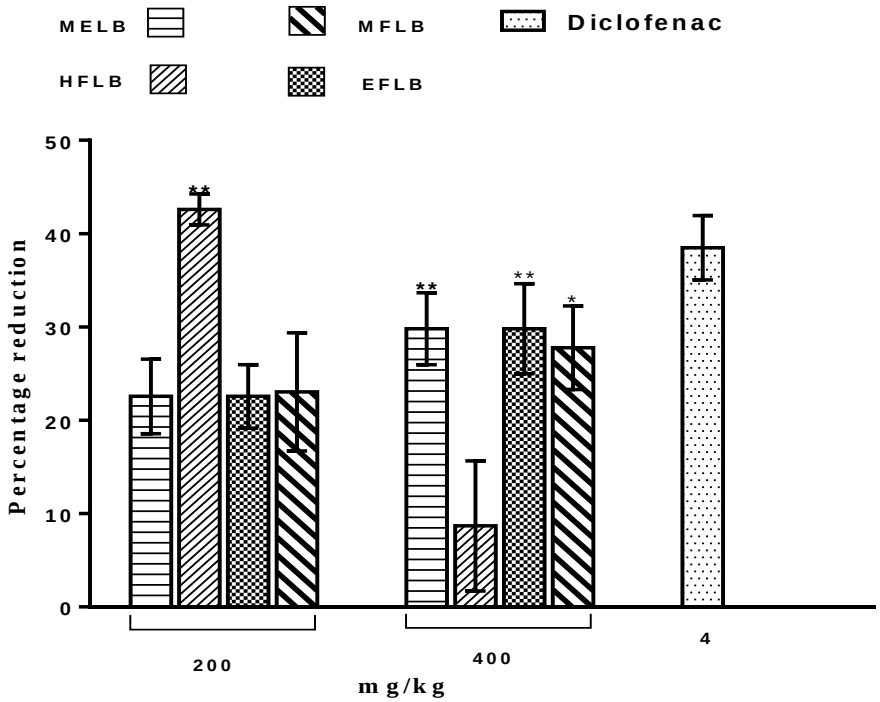
Treatment	Dose (mg/kg)	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h	AUC (ml/h)
		Edema (ml)							
3% Tween 80 (Control)	-	2.15 ± 0.02	2.17 ± 0.02	2.10 ± 0.04	2.00 ± 0.04	1.77 ± 0.04	1.62 ± 0.07	1.53 ± 0.05	13.09
Piroxicam	50	2.05 ± 0.08 (9.1)	2.03 ± 0.05 (12.5)	2.0 ± 0.06 (9.5)	1.85 ± 0.09 (15.8)	1.85 ± 0.08 (11.1)	1.57 ± 0.10 (8.8)	1.47 ± 0.05 (12.5)	12.41
MELB	200	2.17 ± 0.09 (8.9)	2.0 ± 0.13 (24.1)	1.91 ± 0.11 (27.6)	1.81 ± 0.15 (30.5)	1.63 ± 0.12 (33.3)	1.56 ± 0.09 (28)	1.48 ± 0.10 (31)	12.38
	400	2.12 ± 0.15 (13.6)	1.8 ± 0.20 (43.8)	1.86 ± 0.14 (34.2)	1.83 ± 0.07 (30.5)	1.76 ± 0.12 (18.1)	1.78 ± 0.14 (-0.70)	1.50 ± 0.09 (31.3)	12.47
HFLB	400	2.15 ± 0.09	1.98 ± 0.17	1.92 ± 0.17	1.75 ± 0.14	1.73 ±	1.70 ± 0.14	1.52 ± 0.13	12.58

		(11.8)	(28.6)	(29.5)	(40)	0.16 (23.6)	(8.77)	(29.2)	
EFLB	400	1.95 ± 0.06 (7.3)	1.98 ± 0.08 (6.3)	1.95 ± 0.04 (2.9)	1.73 ± 0.08 (15.8)	1.55 ± 0.09 (13.9)	1.38 ± 0.07 (19.3)	1.27 ± 0.05 (29.2)	11.65
MFLB	400	2.17 ± 0.06 (9.1)	2.15 ± 0.07 (4.5)	2.12 ± 0.08 (8.6)	1.85 ± 0.12 (8.4)	1.73 ± 0.12 (-4.2)	1.60 ± 0.09 (-8.8)	1.43 ± 0.08 (6.3)	12.82

n = 6, values shown are mean ± SEM (One Way ANOVA) $p < 0.05$ compared to control. Percentage inhibitions of edema relative to control are in parenthesis. MELB = methanol extract, HFLB = n- hexane fraction, EFLB = ethylacetate fraction, MFLB = methanol fraction, AUC = area under curve

Cotton pellet induced granuloma

The percentage reduction of the granuloma tissue by the extract and fractions was significant ($p < 0.05$) compared to the control. The significant reduction was seen at higher dose of 400 mg/kg of the extract and fractions (Fig 2).



Values are mean ± SEM.; n = 5; *, ** ($p < 0.05$ and $p < 0.01$) respectively; MELB = methanol extract; HFLB = n-hexane fraction; EFLB = ethylacetate fraction; MFLB = methanol fraction.

Figure 2 Effect of extract and fractions of *L. barteri* on cotton pellet-induced granuloma in rats

4. DISCUSSION

It has been shown that three main mediators are responsible for acute and chronic inflammatory reactions induced by egg albumin (Kasahara et al., 2002). The first phase of oedema is attributed to the release of histamine, serotonin or bradykinin by local cells. The second phase which is the late phase is characterized with the release of prostaglandins which strengthens the oedematous condition (Morris, 2003). This indicates that the extract and n-hexane fraction possibly exhibited their anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators (histamine, serotonin and bradykinin) that mediate acute

inflammation induced by egg albumin. Equally, to some large extent, the MELB (200 mg/kg) and EFLB inhibited the late phase of release of prostaglandins that aggravates the inflammatory response in the second phase (Haiping, 2008).

Xylene causes severe vasodilatation and edematous changes of skin as signs of acute inflammation (Kim et al., 2007). The enlarged thickness of ear tissues is as a result of these changes in histopathology. In this study, the extract and fractions significantly inhibited the xylene-induced increase in ear weight. This inhibitory capacity of the extract and fractions can be taken as the evidence of anti-inflammatory effectiveness through the reduction of vasodilatation thus, improving the edematous condition. The cotton-pellet induced granuloma is a known model normally used to evaluate the transudative and proliferative components of the chronic inflammation (Lowry et al., 1951; Castro et al., 1968). In this study, administration of *L. barteri* extract and fractions exhibited good percentage reduction of formed granuloma in a dose-dependent manner which far more exceeds that of diclofenac sodium. The mechanism of action of diclofenac sodium is by inhibition of prostaglandins synthesis at the late phases of inflammation. This effect may be ascribed to the cellular migration and accumulation of collagen, an important mucopolysaccharide to injured sites (Smith and Dewitt, 1995). A decrease in granuloma tissue, prevention of occurrence of the collagen fiber and suppression of mucopolysaccharides are important indicators of the antiproliferative effects of NSAIDs (Verma et al., 2010). In view of this, results of current study demonstrate that the extract and fractions of *L. barteri* have the capacity of inhibiting sub-acute inflammation by interrupting the arachidonic acid metabolism.

The preliminary phytochemical screening of the extract and fractions of *L. barteri* gave positive reaction for tannins, carbohydrate, saponins, diterpenes, flavonoids, alkaloids, resins, glycosides and steroids. Therefore, the anti-inflammatory activity of this plant may be due to the presence of these chemical constituents. Flavonoids are known to inhibit the enzyme prostaglandin synthesis, more specifically the endoperoxide and reported to produce analgesic and anti-inflammatory effect (Alcatraz and Jimenez, 1998; Della Loggia, 1986).

5. CONCLUSION

The study thus, demonstrated the anti-inflammatory activity of the extract and fractions of *Lannea barteri* leaves and found them to be effective in a dose-dependent manner, reduction of edema associated with inflammatory mediators. These activities may be as a result of the phytoconstituents that are present in the plant which may be working either singly or in combination, the action(s) of which improved wound healing, thus, given that scientific evidence to the ethnomedicinal uses of *Lannea barteri*.

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Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

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